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⑤④ **Enzymatic resolution process.**

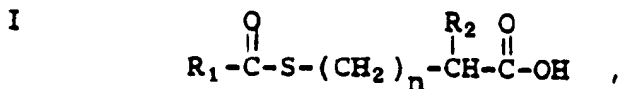
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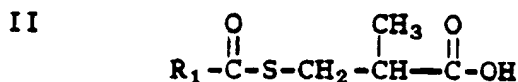
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Description

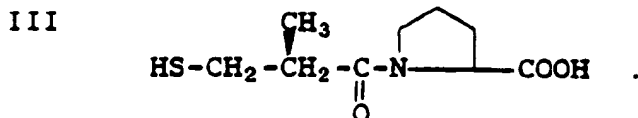
Optically active carboxylic acids represented by the formula



wherein R_1 and R_2 are each independently selected from alkyl, cycloalkyl, aralkyl or aryl, and n is 1 or 2, are useful, for example, as intermediates for the synthesis of various physiologically active materials. For example, a compound of the formula



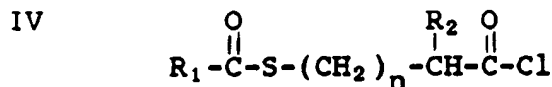
is a key intermediate in the synthesis of 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline (captopril), having the formula



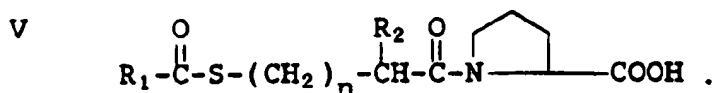
The beneficial activity of captopril depends on the configuration of the mercapto-alkanoyl moiety and the compound of the S configuration is about 100 times more potent than the corresponding R-enantiomer.

Prior art processes for making captopril have utilized chemical and enzymatic resolution procedures. For example, carboxylic acids of formula I are prepared as racemic mixtures which can be separated into the R and S-enantiomeric forms using chemical resolving agents. The so-provided S intermediates can then be used to prepare the desired product. The chemical resolution techniques have the distinct disadvantage, however, that large amounts of very expensive resolving agents are required to make captopril. Additionally, the processes themselves are cumbersome and the yield is relatively low.

Alternatively, racemic compounds of the formula



can be directly coupled to L-proline to produce diastereomers of the general formula



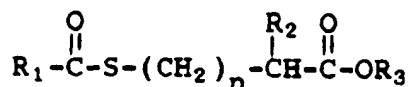
The SS-diastereomer of compound V can be isolated. Subsequent removal of the



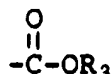
acyl group provides the desired product. However, a drawback to this process is that an equal amount of the RS-diastereomer of compound V is formed which must be discarded. This is highly undesirable in view of the cost of the L-proline.

United States Patent 4,629,701 provides the desired resolved form of the carboxylic acids of formula I by subjecting an ester of the formula

VI



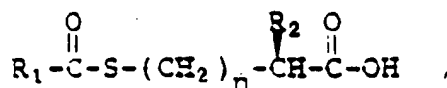
to an enzyme capable of asymmetrically hydrolyzing such an ester. It was found that while the



moiety is hydrolyzed to the acid form, the racemic ester is also resolved into the S or R configuration in improved yields and at lower costs than possible with chemical resolution techniques. However, there is still a considerable expense in making these ester starting materials and higher optical purity is still desired for more active products. Therefore, a process which is less expensive with improved yields and which provides enhanced optical purity would be a useful addition to the art.

In accordance with the present invention, a novel process for preparing optically active compounds of the formula

I'



wherein R₁ and R₂ are each independently selected from alkyl, cycloalkyl, aralkyl or aryl, and n is 1 or 2, is provided. The process comprises treating a racemic mixture of a compound of formula I with an enzyme or microorganism having the ability to asymmetrically hydrolyze the thioester bond of I, in the presence of a solvent.

The following definitions apply throughout this application.

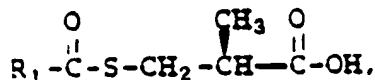
The term "alkyl" as used herein refers to straight or branched chain carbon groups of 1 to 20 carbon atoms, preferably 1 to 6 carbon atoms.

The term "cycloalkyl" as used herein refers to groups containing 5 to 7 carbon atoms.

The term "aryl" as used herein refers to monocyclic or bicyclic aromatic groups containing from 6 to 10 carbon atoms in the ring portion such as phenyl, naphthyl and substituted phenyl or naphthyl containing substituents such as nitro, halogen, methyl or alkoxy groups on the aromatic ring.

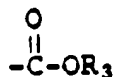
The enzymatic resolution process of the present invention has the advantage that it can provide the desired S-enantiomers of formula I' with optical purity of 95 percent and above at yields exceeding 20 percent. Additionally, because the present process uses a racemic carboxylic acid of formula I as a starting material, instead of the carboxylic acid esters employed in prior art enzymatic processes, there is considerably less expense involved. These and other features make the process of the present invention very attractive for use in preparing optically active compounds of formula I', such as the S-enantiomer of the formula

II'

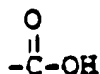


useful in the preparation of captopril.

As discussed above, the prior art enzymatic processes function by the hydrolysis of the carboxy-ester, i.e. hydrolysis of the



moiety in formula VI above to a

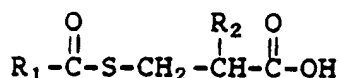


group.

In the present process, the racemic form of a compound of formula I is the starting material. Since this material does not include a carboxy ester, the enzyme or microorganism employed selectively catalyzes the hydrolysis of the thioester bond of one enantiomer of racemic compound I to yield the resolved form of the compounds of formula I' with high optical purity.

Methods for obtaining the racemic starting material of the formula

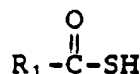
I



are known.

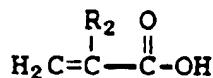
For example, a compound of the formula

VII



can be coupled to a compound of the formula

VIII



in the presence or absence of a suitable solvent, such as hexane, heptane or isopropanol, under the usual conditions for conducting such an addition reaction.

The present process can be carried out in an aqueous solvent, or organic solvent or mixtures thereof. Typical solvents suitable for use in the present process include, but are not limited to, 1,1,2-trichloro-1,2,2-trifluoroethane, toluene, cyclohexane, benzene, deionized water, suitable aqueous buffer solutions and mixtures of these organic and aqueous solvents.

The enzyme or microorganism used in the present process can be any enzyme or microorganism having the ability to asymmetrically hydrolyze thioesters of the general formula I. Various enzymes, such as esterases, lipases and proteases regardless of origin or purity, are suitable for use in the present invention. The enzyme can be in the form of a mixture of animal and plant enzyme, cells of microorganisms, crushed cells or extracts of cells.

Typical genres of microorganism suitable as sources of hydrolyzing enzymes include *Mucor*, *Escherichia*, *Staphylococcus*, *Agrobacterium*, *Rhizopus*, *Aspergillus*, *Nocardia*, *Streptomyces*, *Trichoderma*, *Candida*, *Rhodotorula*, *Torulopsis*, *Bacillus*, *Alcaligenes*, *Pseudomonas*, *Brevibacterium*, *Enterobacter*, *Chromobacterium*, *Arthrobacter*, *Microbacterium*, *Mycobacterium*, *Saccharomyces*,

Penicillium, *Botrytis*, *Chaetomium*, *Ophiobolus*, *Cladosporium* and the like.

Commercially available enzymes suitable for use in the present invention include lipases, such as Amano AY-30 (*Candida cylindracea*), Amano P (*Pseudomonas fluorescens*), Amano N (*Rhizopus niveus*), Amano R (*Penicillium* sp.), Amano FAP (*Rhizopus oryzae*), Amano AP-12 (*Aspergillus niger*),
 5 Amano MAP (*Mucor meihei*), Amano GC-4 (*Geotrichum candidum*), Sigma L-0382 (porcine pancreas), Sigma L-3001 (Wheat germ), Sigma L-1754 (*Candida cylindracea*), Sigma L-0763 (*Chromobacterium viscosum*) and Amano K-30 (*Aspergillus niger*). Additionally, enzymes derived from animal tissue include esterase from pig liver, α -chymotrypsin and pancreatin from pancreas.

Specific microorganisms suitable for use in the present process include *Pseudomonas fluorescens*,
 10 *Pseudomonas putida*, *Pseudomonas ovalis*, *Escherichia coli*, *Staphylococcus aureus*, *Alcaligenes faecalis*, *Streptomyces griseus*, *Streptomyces clavuligerus*, *Nocardia erthropolis*, *Nocardia asteroides*, *Mycobacterium phlei*, *Agrobacterium radiobacter*, *Aspergillus niger*, *Rhizopus oryzae* and the like.

To carry out the process of the present invention, the enzyme and racemic starting material are added to the desired solvent. Typically, the enzyme is adsorbed onto a suitable carrier, e.g. diatomaceous earth
 15 (porous Celite Hyflo Supercel), or the like. This serves the purpose of immobilizing the enzyme which has the effects of controlling the enzyme particle size and preventing aggregation of the enzyme particles when used in an organic solvent. This can be accomplished, for example, by precipitating an aqueous solution of the enzyme with cold acetone in the presence of the Celite Hyflo Supercel followed by vacuum drying. The reaction solution typically contains between about 5 and 200 mg of racemic starting material per ml of
 20 solvent, and preferably contains about 15-50 mg/ml. The enzyme added to the reaction solution may be present in concentrations ranging from about 5 to about 40 mg of enzyme per ml of solvent. While it is desirable to use the least amount of enzyme possible, the amount of enzyme required will vary depending upon the specific activity of the enzyme used.

When the reaction is conducted in an organic solvent, small amounts of water may be added to the
 25 reaction mixture. The water added to the reaction mixture may be present in concentrations ranging from about 0.2 to about 100 mg of water per ml of solvent, or solvent saturated with water, and preferably is present in an amount of about 0.8-5 mg/ml. When an aqueous buffer solution or deionized water is used as the solvent for the reaction, the pH of the reaction solution may be between about 3 and 10, and is preferably maintained at about 5-8 by the addition of suitable materials. Incubation of the reaction solution
 30 can be at a temperature between about 4 and about 60 °C and is preferably carried out at about 30 °C. The reaction time can be appropriately varied depending upon the amount of enzyme used and its specific activity. Typical reaction times for optical purities of 90 percent and above are at least about 5 hour and can range up to about 50 hours for greater conversions and higher optical purities, e.g. optical purities exceeding 95 percent. Optically active *l* can be isolated from the reaction mixture and purified by known
 35 methodologies such as extraction, distillation, crystallization, column chromatography, and the like.

As will be apparent to those skilled in the art, the process of the present invention can be carried out using microbial cells containing an enzyme having the ability to asymmetrically hydrolyze thioesters of the general formula I. When using a microorganism to perform the resolution, the present process is conveniently carried out by adding the cells and the racemic starting material to the desired solvent. Cells
 40 may be used in the form of intact cells, dried cells such as lyophilized, spray-dried or heat-dried cells, immobilized cells, or cells treated with organic solvents such as acetone or toluene. Cells may also be used in the form of treated cell material such as ruptured cells or cell extract.

The present invention will now be described by the following examples, however, it should be understood that the invention is not meant to be limited by the details therein.

45 Example 1

To a solution of racemic 3-acetylthio-2-methylpropanoic acid (405 mg) in 25 ml of 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113) was added 1.0 g of lipase from *Pseudomonas fluorescens* (Amano lipase P-30)
 50 and 90 mg of deionized water. The reaction mixture was shaken on a gyrotary shaker at 280 rpm at 30 °C. The degree of conversion was followed by gas chromatography of reaction mixture filtrates. After 16 hours, the conversion was 84% based on the racemic material initially present. The enantiomeric composition of the remaining unreacted substrate (obtained in 16% reaction yield) was 97.8% S-enantiomer and 2.2% R-enantiomer of 3-acetylthio-2-methylpropanoic acid (enantiomeric excess = 95.6%). In this and all following
 55 examples, the enantiomeric composition of the unreacted 3-acetylthio-2-methylpropanoic acid fraction was determined by capillary gas chromatography following derivatization with thionyl chloride and esterification of the resulting acid chloride with (S)-(+)-2-octanol to form diastereomeric esters which can be separated by capillary GC.

Example 2

10.0 g of lipase from *Pseudomonas fluorescens* (Amano lipase P-30) was added to 50 ml deionized water and the mixture centrifuged for 10 minutes at 1,000 x G. A 40 ml portion of the supernatant was
 5 diluted to 200 ml with deionized water and to this enzyme solution was added 40 g of Celite Hyflo Supercel (diatomaceous earth supplied by Manville Corporation). The mixture was incubated for 3 hours at 28 °C with gentle stirring. The enzyme was then precipitated onto the Hyflo Supercel by slowly adding 300 ml of ice-cold acetone to the mixture while stirring. The mixture was filtered on a sintered glass vacuum filter and the filter cake washed with 300 ml ice-cold acetone. The preparation was dried in a vacuum evaporator at 50 °C
 10 for 18 hours to yield an immobilized enzyme preparation with a water content of 0.45% w/w (Karl Fischer moisture analysis).

A 2 g portion of the above immobilized enzyme preparation was added to a solution of racemic 3-acetylthio-2-methylpropanoic acid (405 mg) in 25 ml of 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113). To this mixture, 60 mg of deionized water was added and the reaction mixture shaken on a gyrotary shaker at 280
 15 rpm at 30 °C. The degree of conversion was followed by high-pressure liquid chromatography (HPLC) of reaction mixture filtrates. After 12 hours, the conversion was 78% based on the racemic substrate initially present. The enantiomeric composition of the remaining unreacted substrate (obtained in 22% reaction yield) was 97.1% S-enantiomer and 2.9% R-enantiomer of 3-acetylthio-2-methylpropanoic acid (enantiomeric excess = 94.2%) as determined by capillary gas chromatography following derivatization.

Example 3

A 2 g portion of the immobilized enzyme preparation described in Example 2 was added to a solution of racemic 3-acetylthio-2-methylpropanoic acid (405 mg) in 25 ml of toluene. To this mixture, 60 mg of
 25 deionized water was added and the reaction mixture shaken on a gyrotary shaker at 280 rpm at 28 °C. After 28 hours, the conversion was 79% complete (HPLC analysis) based on the racemic substrate initially present. The enantiomeric composition of the remaining unreacted substrate (obtained in 21% reaction yield) was 97.5% S-enantiomer and 2.5% R-enantiomer of 3-acetylthio-2-methylpropanoic acid (enantiomeric excess = 95.0%).

Example 4

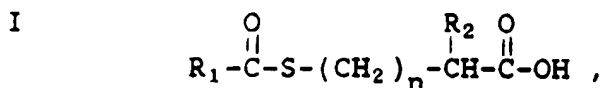
405 mg of racemic 3-acetylthio-2-methylpropanoic acid was dissolved in 15 ml of deionized water, the pH adjusted to 5.0 with 1 N sodium hydroxide, and the solution diluted to 25 ml with deionized water. This
 35 solution was placed in a magnetically stirred pH-stat vessel at 40 °C with pH maintained at 5.0 by the addition of 0.5 N sodium hydroxide. To this solution was added 1.0 g of lipase from *Pseudomonas fluorescens* (Amano lipase P-30) and the degree of conversion followed by HPLC analysis. After 40 hours, the conversion was 76% based on the racemic substrate initially present. The enantiomeric composition of the remaining unreacted substrate (obtained in 24% reaction yield) was 85.7% S-enantiomer and 14.3% R-enantiomer of 3-acetylthio-2-methylpropanoic acid (enantiomeric excess = 71.4%) as determined by
 40 capillary gas chromatography following derivatization.

Example 5

To a solution of racemic 3-acetylthio-2-methylpropanoic acid (405 mg) in 25 ml of 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113) was added 1.05 g of dried mycelia of *Rhizopus oryzae* (ATCC 24563) and 75 mg of deionized water. The reaction mixture was shaken on a gyrotary shaker at 280 rpm at 28 °C. After 69
 45 hours, the conversion was 73% complete (HPLC analysis) based on the racemic substrate initially present. The enantiomeric composition of the remaining unreacted substrate (obtained in 27% reaction yield) was 95.9% S-enantiomer and 4.1% R-enantiomer of 3-acetylthio-2-methylpropanoic acid (enantiomeric excess = 91.8%).
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Claims

1. A process for the selective preparation of a specific enantiomer of a compound of the formula

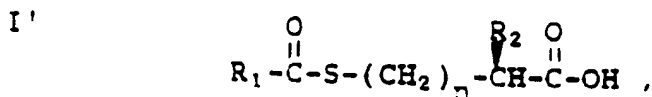


wherein R₁ and R₂ are each independently

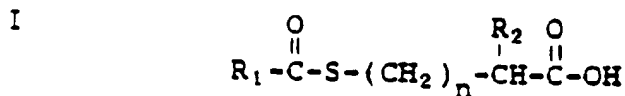
alkyl, cycloalkyl, aralkyl or aryl, and n is 1 or 2;

which process comprises treating a racemic mixture of a compound of formula I with an enzyme, or microorganism containing an enzyme, capable of asymmetrically hydrolyzing the thioester bond of said compound of formula I in the presence of a solvent.

2. The process of claim 1 wherein the enzyme is a lipase, esterase, α-chymotrypsin or pancreatin.
3. The process of claim 1 wherein the microorganism is *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas ovalis*, *Escherichia coli*, *Staphylococcus aureus*, *Alcaligenes faecalis*, *Streptomyces griseus*, *Streptomyces clavuligerus*, *Nocardia erthropolis*, *Nocardia asteroides*, *Mycobacterium phlei*, *Agrobacterium radiobacter*, *Aspergillus niger*, or *Rhizopus oryzae*.
4. The process of claim 1 wherein the solvent is an aqueous solvent, an organic solvent or a mixture thereof.
5. A process for the preparation of optically active compounds having the formula



wherein R₁ and R₂ are each independently alkyl, cycloalkyl, aralkyl or aryl, and n is 1 or 2; which process comprises treating a racemic mixture of a compound of the formula

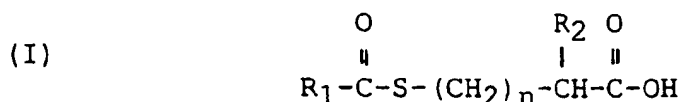


with an enzyme, or microorganism which contains an enzyme, capable of asymmetrically hydrolyzing the thioester bond of said compound of formula I, in the presence of a solvent.

6. The process of claim 5 wherein the enzyme is a lipase, esterase, α-chymotrypsin or pancreatin.
7. The process of claim 5 wherein the microorganism is *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas ovalis*, *Escherichia coli*, *Staphylococcus aureus*, *Alcaligenes faecalis*, *Streptomyces griseus*, *Streptomyces clavuligerus*, *Nocardia erthropolis*, *Nocardia asteroides*, *Mycobacterium phlei*, *Agrobacterium radiobacter*, *Aspergillus niger* or *Rhizopus oryzae*.
8. The process of claim 5 wherein the solvent is an aqueous solvent, an organic solvent or a mixture thereof.

Patentansprüche

1. Verfahren zur selektiven Herstellung eines spezifischen Enantiomers einer Verbindung der Formel



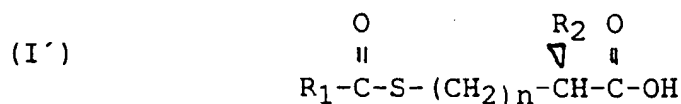
worin R_1 und R_2 je unabhängig Alkyl, Cycloalkyl, Aralkyl oder Aryl sind und n 1 oder 2 ist;
wobei das Verfahren die Behandlung eines racemischen Gemisches einer Verbindung der Formel (I) mit einem Enzym oder einem Mikroorganismus umfaßt, der ein Enzym enthält, das die Thioesterbindung dieser Verbindung der Formel (I) in Gegenwart eines Lösungsmittels asymmetrisch hydrolisieren kann.

2. Verfahren nach Anspruch 1, worin das Enzym eine Lipase, eine Esterase, α -Chymotrypsin oder Pancreatin ist.

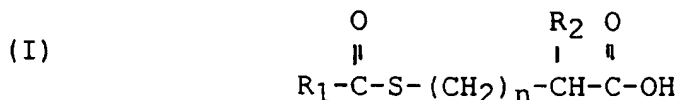
3. Verfahren nach Anspruch 1, worin der Mikroorganismus *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas ovalis*, *Escherichia coli*, *Staphylococcus aureus*, *Alcaligenes faecalis*, *Streptomyces griseus*, *Streptomyces clavuligerus*, *Nocardia erthropolis*, *Nocardia asteroides*, *Mycobacterium phlei*, *Agrobacterium radiobacter*, *Aspergillus niger* oder *Rhizopus oryzae* ist.

4. Verfahren nach Anspruch 1, worin das Lösungsmittel ein wäßriges Lösungsmittel, ein organisches Lösungsmittel oder ein Gemisch davon ist.

5. Verfahren zur Herstellung optisch aktiver Verbindungen der Formel



worin R_1 und R_2 je unabhängig Alkyl, Cycloalkyl, Aralkyl oder Aryl sind und n 1 oder 2 ist;
wobei das Verfahren die Behandlung eines racemischen Gemisches einer Verbindung der Formel



mit einem Enzym oder mit einem Mikroorganismus umfaßt, der ein Enzym enthält, das die Thioesterbindung dieser Verbindung der Formel (I) in Gegenwart eines Lösungsmittels asymmetrisch hydrolisieren kann.

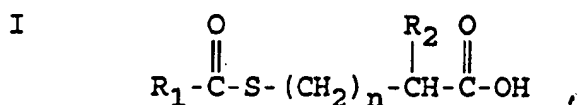
6. Verfahren nach Anspruch 5, worin das Enzym eine Lipase, eine Esterase, α -Chymotrypsin oder Pankreatin ist.

7. Verfahren nach Anspruch 5, worin der Mikroorganismus *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas ovalis*, *Escherichia coli*, *Staphylococcus aureus*, *Alcaligenes faecalis*, *Streptomyces griseus*, *Streptomyces clavuligerus*, *Nocardia erthropolis*, *Nocardia asteroides*, *Mycobacterium phlei*, *Agrobacterium radiobacter*, *Aspergillus niger* oder *Rhizopus oryzae* ist.

8. Verfahren nach Anspruch 5, worin das Lösungsmittel ein wäßriges Lösungsmittel, ein organisches Lösungsmittel oder ein Gemisch davon ist.

Revendications

1. Procédé de préparation sélective d'un énantiomère spécifique d'un composé de formule



dans laquelle R_1 et R_2 sont chacun, indépendamment, un groupe alkyle, cycloalkyle, aralkyle ou aryle, et n est égal à 1 ou 2;

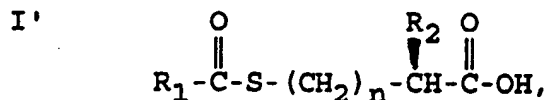
procédé qui consiste à traiter un mélange racémique d'un composé de formule I par une enzyme, ou un micro-organisme contenant une enzyme, capable d'hydrolyser asymétriquement la liaison thio-ester dudit composé de formule I en présence d'un solvant.

2. Procédé selon la revendication 1, dans lequel l'enzyme est une lipase, une estérase, l' α -chymotrypsine ou la pancréatine.

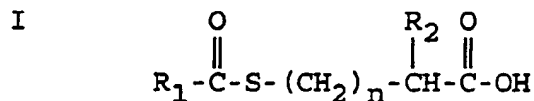
3. Procédé selon la revendication 1, dans lequel le micro-organisme est *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas ovalis*, *Escherichia coli*, *Staphylococcus aureus*, *Alcaligenes faecalis*, *Streptomyces griseus*, *Streptomyces clavuligerus*, *Nocardia erthropolis*, *Nocardia asteroides*, *Mycobacterium phlei*, *Agrobacterium radiobacter*, *Aspergillus niger*, ou *Rhizopus oryzae*.

4. Procédé selon la revendication 1, dans lequel le solvant est un solvant aqueux, un solvant organique ou un mélange des deux.

5. Procédé de préparation de composés optiquement actifs ayant pour formule



dans laquelle R_1 et R_2 sont chacun, indépendamment, un groupe alkyle, cycloalkyle, aralkyle ou aryle, et n est égal à 1 ou 2; procédé qui consiste à traiter un mélange racémique d'un composé de formule



par une enzyme, ou par un micro-organisme contenant une enzyme, capable d'hydrolyser asymétriquement la liaison thio-ester dudit composé de formule I, en présence d'un solvant.

6. Procédé selon la revendication 5, dans lequel l'enzyme est une lipase, une estérase, l' α -chymotrypsine ou la pancréatine.

7. Procédé selon la revendication 5, dans lequel le micro-organisme est *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas ovalis*, *Escherichia coli*, *Staphylococcus aureus*, *Alcaligenes faecalis*, *Streptomyces griseus*, *Streptomyces clavuligerus*, *Nocardia erthropolis*, *Nocardia asteroides*, *Mycobacterium phlei*, *Agrobacterium radiobacter*, *Aspergillus niger*, ou *Rhizopus oryzae*.

8. Procédé selon la revendication 5, dans lequel le solvant est un solvant aqueux, un solvant organique ou un mélange des deux.